REMARKS

I. Status of Claims:

Claims 1, 2, 4, 9, 12, and 15-30 remain pending and under examination in this application. Claims 18, and 19 are amended herein to correct errors in claim dependency. Claims 1, 9 and 30 have been amended to clarify their scope. No new matter is added.

II. Claim Objections:

Claims 18 and 19 are objected to for being dependent from canceled claim 3. *See*, Office Action at page 4. Applicants thank the Examiner for pointing out this issue. Claims 18 and 19 have been amended to depend from claim 1. Accordingly, this objection is moot.

III. Rejections Under 35 U.S.C. § 103(a):

(a) Claims 1, 2, 4, 15-21, 27 and 30 are rejected as allegedly being obvious over Obiakor et al. (*Analytical Biochemistry*, 306:55-62 (2002)) in view of Kotlan et al. (*Immunology Letters*, 65:143-151 (1999)). *See*, Office Action at pages 5-7. Applicants traverse.

The Office Action's reasoning for why it would allegedly have been *prima facie* obvious for the ordinary artisan to combine Obiakor with Kotlan to arrive at Applicants' claimed invention is as follows:

> Obiakor teaches the use of LMD and RT-PCR to determine the sequence of polynucleotides encoding antibodies from suspensions of single cells, where the cells are B cells. The rationale for combining the teachings of Obiakor with the teachings of Kotlan is that both Obiakor and Kotlan teach methods from the same field of endeavor, which is that of the isolation and determination of polynucleotide sequences of immunoglobulins from **single** B cells. The difference between Obiakor and the instant claims is that Obiakor does not teach a lesional tissue, whereas the claims require a lesional tissue. However, because lesional tissues such as medullary breast cancer are known to contain B cells, and because there is reason to isolate and determine the polynucleotide sequences encoding antibodies in B cells infiltrating cancer tissues, and because Kotlan demonstrates that it is possible to isolate and determine the sequence of nucleotides encoding antibodies directed from B cells present in lesional tissue, the use of Obiakor's method with the lesional tissue of Kotlan appears to have a high probability of success. *See*, Office Action, para. bridging pp. 6-7. Emphasis supplied.

The Office Action's stated rationale for combining Obiakor with Kotlan is based on what Applicants believe to be an incorrect understanding of the Kotlan reference. Kotlan is directed to investigating the immunoglobulin (Ig) **repertoire** of B lymphocytes infiltrating a breast medullary carcinoma. To do this Kotlan obtained a tumor sample from a patient, mechanically cut the sample into small bits and treated these bits of tissue with a cocktail of enzymes to break the connections between cells. The product of this enzymatic digestion of the tissue was filtered and then suspended in RPMI 1640 (*see*, p. 144, right column, Materials and methods, Preparation of tumor infiltrating cells). This suspension of all the cells from the filtration procedure is what Kotlan refers to as a "single cell suspension," a term the Examiner appears to believe means a solution in which just one single cell is suspended. Applicants submit that it is clear from a reading of the details of the procedure that Kotlan intended "single cell suspension" to mean simply that the mixture of many cells in the suspension were not attached to each other. Kotlan's single cell suspension would have contained a mixture of many different cells (some B cells and some not). This mixture of suspended cells was used to prepare total RNA, synthesize cDNA, and amplify Ig heavy chain and light chain variable regions by PCR to determine the Ig repertoire of the tumor infiltrating B cells (*see,* Abstract, p. 144, right column, first full para., and page 144, right column, sections 2.2 and 2.3). Since the PCR primers used were specific for Ig variable gene regions (page 144, right column, section 2.3), there was no need to use a cell preparation procedure that excluded non-B cells. Further, since Kotlan's stated purpose was to study the entire repertoire of VH and VL regions of all antibodies expressed by all B cells present in the tumor tissue sample, a method such as Kotlan's that permitted efficient preparation and analysis of the entire repertoire simultaneously in one manipulation is clearly preferable to an approach that requires painstaking isolation of each B cell and individual analysis of that B cell's VH and VL regions, one at a time. The Office Action's rationale for combining the teachings of Obiakor with the teachings of Kotlan is that both Obiakor and Kotlan teach methods from the same field of endeavor: isolation and determination of polynucleotide sequences of immunoglobulins from **single** B cells. In fact, Kotlan's purpose - understanding the **entire repertoire** of Igs present in a breast medullary cancer – was much more efficiently

achieved by the straightforward batch-preparation technique utilized by Kotlan than it would have been by a technique such as Obiakor's that requires sequential isolation and analysis of single cells, one at a time, and concomitant manipulation of vanishingly small quantities of RNA and DNA. If Obiakor's approach was to be used for Kotlan's purpose, Kotlan's efficient batch method would become intolerably time consuming, as single cells would need to be isolated and their DNAs individually analyzed to arrive at an understanding the Ig repertoire in the tissue. The Examiner has suggested no reason one would wish to do that.

It is also noted that there is no motivation to modify Obiakor's method for use with Kotlan's tissue sample. Obiakor's purpose is to study genealogical relationships during clonal expansion and diversification of B lymphocytes as a result of somatic hypermutation or gene conversion (*see*, p. 55, right column, first and second paras.) Somatic hypermutation occurs in B cell germinal centers. That is the reason Obiakor used tissues from appendix and spleen, both of which contain B cell germinal centers. Neither Obiakor nor Kotlan suggests that the B cells in medullary breast cancer are undergoing somatic hypermutation or gene conversion. Thus, modifying Obiakor to use Kotlan's medullary breast cancer tissue instead of appendix or spleen would not serve Obiakor's purpose.

Finally, Applicants' note that the Office Action provides only a conclusory statement regarding a reason one of ordinary skill would wish to modify Obiakor with Kotlan. The Action simply states: "there is reason to isolate and determine the polynucleotide sequences encoding antibodies in B cells infiltrating cancer tissues." The Office Action does not explain what the reason is. Saying that a reason exists, without saying what the reason is, is wholly inadequate to support a *prima facie* case of obviousness. As the U.S. Supreme Court made clear in *KSR Int'l Co. v. Teleflex Inc.*, 127 S.Ct. 1727, 1741 (2007), "[R]ejections on obviousness grounds cannot be sustained by mere conclusory statement; instead, there must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness." Merely saying "there is reason" plainly does not satisfy that requirement.

At least for the foregoing reasons, Applicants respectfully request that this rejection under 35 U.S.C. § 103(a) be reconsidered and withdrawn.

(b) Claims 9, 12, 28 and 29 are rejected as allegedly being obvious over Obiakor et al. in view of Zhang et al. (*Cancer Research*, 55:3584-3591 (1995)). *See*, Office Action at pages 7-9. Applicants traverse.

The Office Action states, in relevant part:

> It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have used the method of Obiakor for the purpose of obtaining polynucleotides encoding antitumor antibodies from a B cell found in lesional tissue such as the melanoma tissue of Zhang, because Obiakor teaches the use of LMD and RT-PCR to determine the sequence of polynucleotides encoding antibodies from suspensions of single cells, where the cells are B cells, and because Zhang teaches that infiltrating B cells may be a source of anti-tumor antibodies (page 3585, left column). Zhang uses a different method for the isolation of tumor infiltrating B cells than that recited in the claims. However, as discussed above Obiakor provides evidence that the method of isolating a single B cell from a tissue using LMD is known in the art. Furthermore, because Zhang teaches that one goal of isolating infiltrating B cells from a tumor tissue is to detect anti-tumor antibodies and to use the polynucleotides encoding the antitumor antibodies in methods of making therapeutic anti-tumor antibodies, one would have been motivated to use the method of Obiakor as a method of isolating single B cells because the method of Obiakor ensures the detection of a single species of antibody in one isolation step. Thus, Zhang's method would be improved because Zhang's method requires more than one step to arrive a single monoclonal B cell population. *See*, Office Action, p. 9.

Zhang is directed to a method of making specific antitumor antibodies from tumor infiltrating B lymphocytes (TIL-Bs) in a melanoma in order to use them to identify and characterize tumor antigens. The process involves preparing suspensions of TIL-Bs from surgical biopsies and expanding these cells in the presence of EBV in order to transform the TIL-Bs. Antibody-containing supernatants from the EBV-transformed cells are assayed against tumor cell lines to identify tumor-specific B cell lines (*see*, Materials and Methods, page 3585).

Obiakor is directed to studying genealogical relationships during clonal expansion and diversification of B lymphocytes as a result of somatic hypermutation or gene conversion in appendix and splenic germinal centers (*see*, p. 55, right column, first and second paras.).

There is no motivation to combine Obiakor and Zhang. Rather than seeking to examine a cross-section of all B cells present in a tissue, as did Obiakor, Zhang's purpose was to identify, out of all the B cells present in the tissue, a particular subset of B cells: those that produce tumor specific antibodies. Trying to do this by randomly picking out and studying individual B cells

would have been a very haphazard way to reach the goal. The method actually used by Zhang allowed him to pres-select those B cell lines that express the tumor-specific antibodies of interest, prior to doing the work of analyzing their antibody genes. Zhang's method involves a step of screening the antibody-containing supernatants of TIL-B cell lines against tumor cell lines (presumably carrying tumor antigens of interest) to identify the subset of TIL-B cell lines to be pursued. Obiakor does no such thing. Obiakor's method would involve painstaking isolation by LMD of single cells, isolation of their DNA, expression of the DNA in a cell, and only then testing the antibodies produced by those cells to determine if they bind tumor antigens. In contrast, Zhang, isolates and pursues only the "positives" – the cell lines that secrete antibodies binding tumor antigens. Obiakor's method provides no indication of the antibody specificity of B cells in a tissue and effectively precludes any sort of preselection of B cells secreting useful antibodies, out of all of the B cells present in the tissue. While the Office Action seems to assume that every B cell present in a tumor will bind to a tumor-specific antigen (see the statement regarding detection of an antibody "in one isolation step"), there is no evidence to support this assumption. Since it is possible that a large majority of the B cells in a lesional tissue may not bind tumor antigens (and neither Zhang nor Obiakor teaches otherwise), Zhang's preselection technique would seem to make much more sense for identifying tumor-antigen-specific B cells than does Obiakor's brute-force technique. In the absence of any teaching or suggestion regarding this matter, there is simply no reason to modify Zhang with Obiakor's method.

At least for the foregoing reasons, Applicants respectfully request that this rejection under 35 U.S.C. § 103(a) be reconsidered and withdrawn.

(c) Claims 1, 2, 4, 15-23, 25 and 30 are rejected as allegedly being obvious over Obiakor et al. in view of Walton et al. (*Atherosclerosis*, 135:65-71 (1997)). *See*, Office Action at pages 9-10. Applicants traverse.

The Office Action states, in relevant part:

> it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have used the method of Obiakor for the purpose of obtaining

> polynucleotides encoding the polynucleotides encoding antibodies from a B cell found in lesional tissue such as inflammatory atherosclerotic lesion, because Obiakor teaches the use of LMD and RT-PCR to determine the sequence of polynucleotides encoding antibodies from suspensions of single cells, where the cells are B cells to obtain monoclonal antibody species, and Walton teaches the desirability of isolating such antibodies for identification of tissue antigens. Furthermore, because Walton teaches that one goal of isolating antibody-encoding polynucleotides from atherosclerotic lesions is to tissue autoantigens, one would have been motivated to use the method of Obiakor as a method of isolating B cells because the method of Obiakor ensures the detection of a single species of antibody in one isolation step. *See*, Office Action, p. 10, last para.

The Office Action's combination is based on an improper reading of Walton, specifically that "Walton teaches the desirability of isolating such antibodies [i.e., antibodies from inflammatory atherosclerotic lesions] for identification of tissue antigens." In fact, Walton's ultimate results teach the opposite. See, e.g., the last sentence of Walton's abstract.

Walton is directed to investigating whether inflammation in atherosclerotic abdominal aortic aneurysms (AAA) is a response to specific antigens generated by disease in the aortic wall. Walton does this by examining the **repertoire** of immunoglobulin heavy chain genes present in DNA extracted from cells in the outer walls of AAA. Walton expected to find a restricted usage of Ig VH genes that would indicate an antibody response to a limited set of antigens. Instead, Walton found that the usage of Ig heavy chain genes was unrestricted, suggesting that in atherosclerotic AAA, the B lymphocytes in the outer aneurysm wall are not clonal populations generated in response to either a single or a limited number of specific tissue antigens.

It is true that Walton states that "[o]ne important advantage of using the VH gene usage to demonstrate a monoclonal or oligoclonal response is the potential to clone specific immunoglobulin genes and subsequently identify the tissue antigens and their autoantigenic domains." See, p. 69, right col., first sentence of first full para. However, this sentence must be read in context of the reference as a whole. Applicants draw the Examiner's attention to the paragraph bridging the left and right columns of p. 70, where Walton teaches:

> ...the unrestricted usage of immunoglobulin heavy chain genes in atherosclerotic AAA indicates a polyclonal response. This **precludes** the cloning of immunoglobulin heavy chain genes to identify specific antigens and indicates that the clonal expansion in the aortic adventitia of B cells specific to numerous antigens is likely to be a constant feature of the inflammatory process. (Emphasis supplied.)

In other words, although Walton suggests that a theoretical advantage of analyzing VH gene usage to demonstrate a monoclonal or oligoclonal response is the potential to clone specific immunoglobulin genes and to identify the antigens they bind, Walton is unequivocal that in atherosclerotic AAA, where usage of immunoglobulin heavy chain genes turns out to be unrestricted, cloning of immunoglobulin heavy chain genes to identify specific antigens is "precluded." See, Abstract, final sentence. Thus, the alleged motivation to combine Obiakor and Walton to arrive at Applicants' claimed invention does not exist. In fact, Walton clearly teaches away from further attempts to study this question, whether by Walton's technique or any other.

At least for the foregoing reasons, Applicants request that this rejection under 35 U.S.C. § 103(a) be reconsidered and withdrawn.

(d) Claims 1, 2, 4, 15-21, 23-26 and 30 are rejected as allegedly being obvious over Obiakor et al. in view of Mallison et al. (*Infection and Immunity*, 59(11):4019-4025 (1991)). *See*, Office Action at page 11. Applicants traverse.

The Office Action states, in relevant part:

> The methods of Obiakor may be used to study lesions such as those taught by Mallison. Mallison shows that in models of periodontal disease there is an influx of B cells in cases where there is chronic inflammation Mallison teaches that more study is needed to understand the pathological processes associated with periodontal disease (page 4024).
>
> Therefore, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have used the method of Obiakor to isolate single B cells and further to isolate and obtain polynucleotides encoding antibodies for the further understanding of pathological processes associated with periodontal disease. One would have had a reasonable expectation of success in using the method of Obiakor with the tissues of Mallison because Mallison demonstrates that B cells are present. *See*, Office Action, p. 11, last two paras.

As a preliminary matter, Applicants note that the Office Action's rationale for combining Mallison with Obiakor is that Mallison teaches that more study is needed to understand the pathological processes associated with periodontal disease. Mallison did outline some unknown areas in this field (most of which have little or nothing to do with the specific antibodies secreted by B cells) and concluded with "We look forward with interest to studies addressing these

issues," but such a vague statement can hardly be said to be a motivation to utilize any given technique, much less one designed for an entirely different purpose.

Mallison sought to understand the pathological process associated with periodontal disease. Specifically, Mallision was focused on determining why chronic inflammation, but not acute inflammation, leads to local accumulation of plasma cells. To determine this, Mallison used a rabbit model of inflammation and showed that plasma cell accumulation requires the concurrent presence of chronic inflammation, activators of microbial origin and specific antigen. There is no teaching or suggestion whatsoever in Mallison regarding the need or desirability to isolate a single B cell from the lesional tissue and to obtain a polynucleotide encoding an antibody heavy chain and a polynucleotide encoding an antibody light chain of the isolated B cell. This is so because Mallison already knows the antigen – in fact, Mallison selected the antigen (horseradish peroxidase) – for his model. Rather than attempting to isolate B cells to identify the immunoglobulin sequences and identify the antigen they bind, Mallison is attempting to understand what factors contribute to the accumulation of plasma cells in the vicinity of chronic inflammation induced by horseradish peroxidase. See, abstract. It is difficult to see in Mallison any reason whatsoever one would wish to study the antigen specificity of individual B cells, whether by Obiakor's method or any other.

At least for the foregoing reasons, Applicants request that this rejection under 35 U.S.C. § 103(a) be reconsidered and withdrawn.

CONCLUSION

For the reasons set forth above, Applicants submit that all grounds for rejection have been overcome and that all of the pending claims are now in condition for allowance, which action is requested.

In the event that a telephone conversation could expedite the prosecution of this application, the Examiner is requested to call the undersigned at the number provided below.

Applicants petition for a three month extension of time to respond to the outstanding Office Action. Other than the extension fee and the fee associated with the filing of the IDS

being submitted herewith, no other fees are believed to be due. However, if Applicants are mistaken, please apply any required charges or credits to Deposit Account No. 06-1050, referencing Attorney Docket No. 14875-0144US1.

Respectfully submitted,

Date: January 25, 2010

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